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## **PROCEEDINGS OF THE NUTRITION SOCIETY**

### **ABSTRACTS OF COMMUNICATIONS**

*The Four Hundred and Ninth Meeting of the Nutrition Society (One Hundred and Sixty-second of the Scottish Group) was held in the School of Agriculture, University of Aberdeen on Thursday and Friday, 21/22 March 1985, when the following papers were read:*

**Kinetics of glucose metabolism in newborn lambs.** By J. C. HODGSON and D. J. MELLOR, *Moredun Research Institute, 408 Gilmerton Road, Edinburgh EH17 7JH*

Newborn lambs must initiate and sustain heat production in order to survive. Deaths from hypothermia are common yet no quantitative information on substrate use during the first hours of life is available. Kinetics of glucose metabolism and the effects of dietary restriction and ambient temperatures were therefore determined in lambs up to 17 h old.

Four pairs of Dorset lambs (3–5.5 kg) were held at 5 or 30° air temperature and offered ewe colostrum at regular intervals from 2 or 5 h of age. At 2.5 h of age a single 'shot' of [2-<sup>3</sup>H]glucose was given intravenously and the decline in plasma glucose specific radioactivity followed for 2.5 h, and the total entry rate and pool size of plasma glucose calculated (see Table). The procedure was repeated at 8 and 14 h of age.

Ambient temperature (°)	Age at first feed (h)	Age (h)...	Total glucose entry rate (mg/min per kg)			Pool size (mg/kg)		
			2.5–5	8–10.5	14–16.5	2.5–5	8–10.5	14–16.5
5	5		7.3	12.7	13.6	332	725	639
30°	5		7.4	11.1	8.9	205	417	234
5	2		13.3	14.6	14.9	751	1185	646
30°	2		9.7	10.3	13.1	348	492	491
Pooled SE			1.30	1.59	1.38	34.4	89.1	30.8

In unfed lambs there was no effect of temperature on the mean total entry rate of glucose. This may indicate that a rate of about 7.5 mg/min per kg was the maximum possible or that it remained low in lambs at 5° through a 'sparing' action of increased lipid metabolism.

The effect of feeding on the glucose entry rate tended to be greater, and for pool size was significantly greater ( $P < 0.05$ ), at 5 than at 30°. Higher rates at 30° coincide with higher basal metabolic rates observed after feeding (Eales & Small, 1981) and, for lambs at 5°, suggest the removal of substrate limitation to glucose production. Glucose entry rates similar to those in lambs fed from 2 h-old were achieved by 8–10.5 h in those fed from 5 h and were sustained in all lambs apart from those fed from 5 h and kept at 30°. Pool sizes fell between 8–10.5 h and 14–16.5 h in all lambs, apart from those fed from 2 h and kept at 30°, suggesting depletion of glucose reserves. This was most severe in lambs fed from 5 h and kept at 30° and in those fed from 2 h and kept at 5°.

The entry rates reported here for fed lambs are similar to the mean rate of 18.6 mg/min per kg observed in fetal lambs aged 124–134 d (Hodgson *et al.* 1980) and to other published values of 11.5–14.7 mg/min per kg for fed lambs aged 1–9 d.

Eales, F. A. & Small, J. (1981). *Research in Veterinary Science* **30**, 266–269.

Hodgson, J. C., Mellor, D. J. & Field, A. C. (1980). *Biochemical Journal* **186**, 739–747.

**The distribution of triglycerides in the milk fat of Friesian and Jersey cows.** By J. L. CLAPPERTON, W. BANKS and W. STEELE, *The Hannah Research Institute, Ayr KA6 5HG*

Published results for the triglyceride distribution in bovine milk fat show bimodality, with maxima occurring at about carbon number (CN) 38 and CN 50, and a minimum at CN 44 (Badings *et al.* 1983; Banks *et al.* 1984; Von Precht & Peters, 1984).

However, in a recent experiment, in which four Friesian or four Jersey cows were given a basal diet of hay, sugar-beet pulp and barley-based concentrates, unimodal distributions were obtained for the milk fat triglycerides (see Table). When soya-bean oil (500 g/d) was added to the diet, the milk fat of both breeds exhibited bimodal triglyceride distributions (see Table).

CN	Milk-fat triglycerides (mmol/mol)			
	Friesian		Jersey	
	No oil	Soya-bean oil	No oil	Soya-bean oil
28	7	6	11	6
30	14	11	11	9
32	35	28	25	22
34	84	62	61	60
36	139	115	114	107
38	137	136	150	133
40	106	112	124	109
42	93	77	101	80
44	86	70	96	73
46	84	75	88	78
48	83	86	80	89
50	77	99	72	106
52	44	83	49	88
54	11	40	18	40

Thus the form of the triglyceride distribution is governed largely by basal diet.

Badings, H. T., Schaap, J. E., De Jong, C. & Hagedoorn, H. E. (1983). *Milchwissenschaft* **39**, 156–161.

Banks, W., Clapperton, J. L., Girdler, A. K. & Muir, D. D. (1984). *Proceedings of the Nutrition Society* **43**, 105A.

Von Precht, D. & Peters, K.-H. (1984). *Milchwissenschaft* **39**, 652–656.

**The effect of supplementation with concentrates on the utilization of hay from unimproved upland pasture.** By A. J. VERA, R. F. E. AXFORD and R. A. EVANS, *Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Gwynedd*

To a ration consisting of poor quality hay fed *ad. lib.* (H), supplements were added at two levels. The supplements were rolled barley fed at 100 g/d (B1) and 200 g/d (B2) with or without added urea at 10 g/d (C1) or 20 g/d (C2) as shown in the Table. These five rations were allocated to four sheep, fitted with duodenal cannulas, according to a Youden square design. Samples of food, residues, duodenal digesta, faeces and urine were collected over the last 4 d of each 7-d period and analysed for dry matter (DM), organic matter (OM), gross energy (GE), total nitrogen (TN) and non-ammonia N (NAN).

Diet . . .	H	B1	B2	C1	C2	SE
<b>Intake</b>						
DM: Hay (g/d)	785.8	683.9	794.6	734.2	766.7	31.1
Barley (g/d)	—	87.4	174.9	87.4	174.9	
Urea (g/d)	—	—	—	10	20	
GE (MJ/d)	15.47	15.1	18.8	16.2	18.5	0.6
TN (g/d)	11.38	11.64	15.31	16.24	22.40	0.5
<b>Digesta flow to duodenum</b>						
GE (MJ/d)	9.87	9.64	11.55	10.60	10.78	0.5
NAN (g/d)	14.66	14.16	18.21	16.63	18.01	1.1
<b>Faecal output</b>						
GE (MJ/d)	7.97	8.2	9.4	8.8	8.0	0.3
TN (g/d)	6.32	6.13	7.15	6.7	6.9	0.3

The digestibility in the stomach of the GE of the diets containing the higher levels of barley (B2 and C2) was 39.8%, which was significantly higher than that of the other diets which averaged 35.0%.

For all diets, except for diet C2, there was evidence of recycling of N with net increases across the stomach. The flows of NAN into the duodenum were higher for diets B2 and C2 (18.1 g/d) than for diets H, B1 and C1 (15.1 g/d).

The digestibility of the hay in the supplemented rations was estimated assuming that the added urea was completely hydrolysed in the rumen and that the digestibility of the barley conformed constantly to published values. It was found that the overall digestibility of GE of the hay was depressed in diets B1, B2 and C1 and restored in diet C2. The overall digestibility of TN of the hay was reduced at the lower levels of supplementation (B1, C1). The addition of supplements did not increase the intake of hay.

It seems that there may be a threshold value for a concentrate supplement to be effective.

**Effect of oestrogenic and androgenic compounds on growth and body composition of male castrate lambs.** By S. B. SINGH, H. GALBRAITH and J. R. SCAIFE, *School of Agriculture, 581 King Street, Aberdeen* and E. A. HUNTER, *AFRUS, Kings Building, University of Edinburgh, Edinburgh*

Combinations of oestrogenic and androgenic compounds are considered to be effective in stimulating growth in castrate male ruminants (e.g. Heitzman, 1981).

We investigated the effects of the naturally occurring androgen testosterone (T) (Intervet Laboratories Ltd) and the synthetic steroid trenbolone acetate (F) ('Finaplix', Hoechst UK) combined or not with the naturally occurring oestrogen oestradiol-17 $\beta$  (O) ('Compudose' slow-release formulation, Elanco Products Ltd). The compounds were given by subcutaneous implantation 49 d before slaughter. Forty-eight Greyface wether lambs (mean live weight 32 kg) were allocated to treatments as follows: sham-implanted controls (C), 40 mg F (F), 50 mg T (T), 15 mg O (O), 40 mg F+15 mg O (FO), 50 mg T+15 mg O (TO). The lambs were offered to appetite a diet containing 12.5 MJ metabolizable energy and 24.2 g nitrogen/kg dry matter. The results, which are mean values for each group, were evaluated by analysis of variance for treatment effects and interactions.

Treatment group . . .	C	O	F	T	FO	TO	SED
Live-weight gain (LWG) kg/d	0.30	0.44	0.31	0.38	0.42	0.44	0.033
Dry matter intake (DMI) kg/d	1.39	1.55	1.38	1.43	1.47	1.54	0.06
Food conversion efficiency*	4.72	3.62	4.45	3.97	3.56	3.59	0.22
Empty body-weight (EBW) kg	40.8	45.5	40.2	43.0	44.7	45.8	1.52
Carcass weight kg	22.2	24.3	21.6	22.9	24.0	24.4	0.84
Carcass:							
Dry matter (kg)	10.8	11.4	10.6	10.5	11.5	11.1	0.51
Crude protein (kg)	3.32	3.70	3.25	3.41	3.69	3.81	0.15
Lipid (kg)	4.11	4.60	4.07	4.42	3.63	3.60	0.50
Liver (g/kg EBW)	21.3	24.6	22.4	22.5	22.9	23.0	1.05
Kidneys (g/kg EBW)	2.89	2.91	3.01	2.92	3.06	2.76	0.15

\*DMI/LWG.

Treatment O resulted in faster growth, greater food intake, better food conversion efficiency (FCE), heavier empty body and carcass weights, a greater total weight of carcass dry matter and crude protein ( $N \times 6.25$ ), and liver weight. T significantly improved FCE, but F had no significant effect on growth and body characteristics. None of the treatments altered the proportion of water, crude protein or fat in the carcass.

Neither T nor F, when combined with O, had growth effects additive to those produced by O given alone. This result is at variance with that suggested to occur in castrate male ruminants treated with a combination of androgenic and oestrogenic compounds (e.g. Heitzman, 1981). Possible reasons may involve the dose levels and formulation of the products used.

Heitzman, R. J. (1981). In *Hormones and Metabolism in Ruminants*, pp. 129-138. [J. M. Forbes and M. A. Lomax, editors]. London: Agricultural Research Council.

**Effect of oestrogenic and androgenic compounds on some endocrine and body characteristics of male castrate lambs.** By H. GALBRAITH, S. B. SINGH, J. R. SCAIFE and G. D. HENDERSON, *School of Agriculture, 581 King Street, Aberdeen*

Certain compounds with oestrogenic and androgenic activity are widely used alone, or in combination, to stimulate growth in farm animals (e.g. Galbraith & Topps, 1981). Measurements of concentrations of these compounds in blood and their effects on concentrations of endogenous hormones and on tissues sensitive to sex hormones are important in providing information on their biological activity within animals. The compounds studied were the androgens testosterone (T) and trenbolone acetate (F) and the oestrogen oestradiol-17 $\beta$  (O), administered 49 d before slaughter to forty-nine Greyface male castrate sheep (mean live weight 32 kg) as described by Singh *et al.* (1985). The treatments were sham-implanted controls (C), 40 mg F (F), 50 mg T (T), 15 mg O (O), 40 mg F+15 mg O (FO), 50 mg T+15 mg O (TO). Selected results are shown in the Table.

Treatment group . . .	C	O	F	T	FO	TO	SED
Thyroid glands (mg/kg empty body-weight (EBW))	90.7	73.8	64.4	81.3	70.6	89.4	7.64
Pancreas (g/kg EBW)	1.00	1.45	1.25	1.10	1.27	1.11	0.18
Thymus (g/kg EBW)	2.21	2.20	1.38	2.39	2.39	2.10	0.30
Penile tissue (g/kg EBW)	0.63	0.59	1.22	1.00	0.94	0.92	0.10
Teat length (mm)	8.17	12.1	5.74	8.50	10.2	10.8	0.86
Blood plasma samples*:							
Insulin (ng/ml)	1.30	3.22	0.70	1.62	1.75	2.08	0.47
Thyroxine (ng/ml)	139	54.9	110	103	57.9	83.9	23.7
O (pg/ml)	4.8	131	7.2	3.4	46.9	46.9	24.4
F (ng/ml)			1.13		1.32		0.27
T (ng/ml)				1.45		2.28	0.68

\*Samples taken at 10.00–11.00 hours at weekly intervals: only values at 5 weeks are presented.

O concentrations in blood were lower in treatments TO and FO than in treatment O. However, mean values for T and F in blood were relatively unaffected by the presence of O in the combined, compared with single treatments. The main effects due to O were an increased teat length (oestrogenic effect), a tendency to increase pancreas weight and plasma insulin concentrations and to reduce the weight of the thyroid glands and plasma thyroxine concentrations. F increased the weight of penile tissue (androgenic effect). The reduction in thymus weight due to F alone was reversed in the presence of O (FO) but was not induced by T.

The results, which indicate the presence of biologically active levels of all of the implanted steroids, should be considered in the context of the growth stimulation produced by O, and T to a limited extent, but not by F (Singh *et al.* 1985).

Galbraith, H. & Topps, J. H. (1981). *Nutrition Abstracts Review, Series B* 51, 521–540.  
Singh, S. B., Galbraith, H., Scaife, J. R. & Hunter, E. A. (1985). *Proceedings of the Nutrition Society* 44, 93A.

**Influence of oestrogenic and androgenic compounds on blood lipids, subcutaneous fatty acid composition and the activities of certain lipogenic enzymes in sheep.** By S. B. SINGH, J. R. SCAIFE, H. GALBRAITH and C. JESSIMAN, *School of Agriculture, 581 King Street, Aberdeen*

In ruminants the use of oestrogenic and androgenic compounds as growth promoters can cause changes in the deposition of protein and fat. In wether lambs, a combination of the natural oestrogen, oestradiol-17 $\beta$ , and the synthetic androgen, trenbolone acetate, influence blood lipid concentration and the activity of fatty acid synthetase in liver and adipose tissue (Burch *et al.* 1982; Scaife *et al.* 1982). The work presented here reports the effects of administration of 15 mg oestradiol-17 $\beta$  (O), 40 mg trenbolone acetate (F) and 50 mg testosterone (T), given alone or in combinations (FO and TO), upon blood lipid concentration, subcutaneous fatty acid composition and the activities of certain lipogenic enzymes in wether sheep.

Weekly blood samples were obtained for the analysis of plasma free fatty acids (FFA), triglycerides and cholesterol. Samples of subcutaneous adipose tissue (SCAT) for fatty acid analysis were taken at slaughter. Separate samples of SCAT and liver were taken for enzyme assay but only results from groups C (control), O, F, T and FO are available.

Neither treatments O, F or FO had any significant effect on plasma FFA but treatment T significantly increased the concentration of plasma FFA between weeks 2 and 7. Plasma cholesterol levels increased gradually in all treatment groups but in animals receiving treatment O, cholesterol levels were significantly higher 2-3 weeks after implantation.

Acetyl-CoA carboxylase (AcCBX; EC 6.4.1.2) activity was not significantly affected by hormone implantation. The mean values for fatty acid synthetase (FAS) activity in liver tended to increase due to treatments O and F. In adipose tissue, treatment O significantly increased FAS activity, however, the effects of O were reduced when given in the combination FO. The activities of isocitrate dehydrogenase (NADP<sup>+</sup>) (ICDH; EC 1.1.1.42) and ATP-citrate (*pro*-3S) lyase (CL, EC 4.1.3.8) in liver were not markedly affected by steroid treatment but in adipose tissue, activities were significantly altered on treatment T.

Enzyme	Treatment group . . .	Enzyme activity (nmol/min per mg protein)					SED
		C	O	F	T	FO	
AcCBX	Liver	1.19	1.09	1.55	0.95	0.93	0.24
	SCAT	6.61	9.72	7.18	7.0	6.74	1.84
FAS	Liver	0.19	0.37	0.28	0.24	0.22	0.08
	SCAT	8.6	21.7	11.0	7.88	16.00	5.40
ICDH	Liver	157	127	135	144	169	33.8
	SCAT	89	126	160	143	125	54.8
CL	Liver	1.45	2.61	1.90	2.18	2.28	1.38
	SCAT	1.14	0.90	2.12	0.29	0.60	0.71

Treatments O, T and TO tended to lower the ratio of the fatty acids C18:0/C18:1 + C18:2 + C18:3 in subcutaneous adipose triglycerides. The results confirm that androgenic and oestrogenic compounds influence total lipid deposition and metabolism in ruminants.

Burch, L., Scaife, J. R. & Galbraith, H. (1982). *Hormone Metabolism Research* 14, 52-53.

Scaife, J. R., Shehab-Eldin, F. M. & Galbraith, H. (1982). *Hormone Metabolism Research*

14, 589-592.

**Protein turnover in preterm infants by determining the enrichment of  $^{15}\text{N}$  in ammonia: a comparison of tracers.** By T. A. STACK<sup>1</sup>, T. PRESTON<sup>2</sup>, P. J. REEDS<sup>3</sup>, D. J. LLOYD<sup>1</sup> and P. J. AGGETT<sup>1</sup>, <sup>1</sup>*Department of Child Health, University of Aberdeen, Foresterhill, Aberdeen*, <sup>2</sup>*Scottish Universities Research and Reactor Centre, East Kilbride, Glasgow*, <sup>3</sup>*Rowett Research Institute, Bucksburn, Aberdeen*

Protein turnover rates in male, preterm, low-birth-weight infants were derived and compared using [ $^{15}\text{N}$ ]glycine and hydrolysed, nucleic acid-free,  $^{15}\text{N}$ -labelled yeast protein as tracers and using a less invasive methodology than other current techniques.

Seven infants were studied, one (ND) was studied twice; four were given formula feeds (LBW SMA; 3340 kJ/l; 20 g protein/l) and three were given their own mother's milk. All infants had regained their birth weights and were gaining weight. The  $^{15}\text{N}$  tracers were given as a single nasogastric infusion on a randomized basis about 45 min before a feed on days 1 and 3 of a 3 d metabolic balance study. Protein turnover was measured from the  $^{15}\text{N}$  enrichment of nitrogen in urinary ammonia over periods of about 12 h (Waterlow *et al.* 1978). Between each dose, urine for background [ $^{15}\text{N}$ ]ammonia enrichment was obtained after 33 h. Urine samples were acidified with sulphuric acid and stored at  $-20^{\circ}$ . The dose of [ $^{15}\text{N}$ ]glycine (96 atoms %) ranged from 0.609 to 0.964 mg  $^{15}\text{N}$  and, for the yeast (92 atoms %), the dose ranged from 0.918 to 1.493 mg.

*Protein flux (Q), synthesis (S) and breakdown (B) (g/kg per d)*

Diet	Infant	Q yeast	Q glycine	S yeast	S glycine	B yeast	B glycine
Formula feed	I.L.	17.94	12.44	15.87	10.47	15.25	9.89
	R.D.	16.75	11.5	16.18	10.97	14.26	9.1
	N.D. (1)	32.56	15.13	32.05	14.68	30.19	12.88
	N.D. (2)	33.19	15.44	32.68	14.91	30.75	12.92
	R.A.	17.0	14.50	16.4	13.88	14.86	11.77
Human milk	D.D.	19.13	16.25	18.73	15.85	17.32	14.41
	G.D.	14.75	18.31	14.12	17.63	12.94	16.38
	J.K.	11.13	8.44	10.77	8.11	9.38	6.79
	Mean (n 7)	18.5	13.8	17.8	13.1	16.4	11.6
	SD	6.85	3.29	6.88	3.36	6.69	3.29

The results (see Table) derived from  $^{15}\text{N}$ -labelled yeast protein were higher than those derived from [ $^{15}\text{N}$ ]glycine. [ $^{15}\text{N}$ ]glycine gave protein synthesis rates similar to those obtained by other workers using [ $^{13}\text{C}$ ]leucine (de Benoist *et al.* 1984) but, in contrast to earlier reports (Pencharz *et al.* 1983), the three infants given human milk did not have higher synthesis rates based on [ $^{15}\text{N}$ ]glycine than the formula-fed infants.

This methodology may be useful in studies of protein turnover in older children on a day-case basis as well as in neonates.

The authors thank Dr D. Halliday for the gift of  $^{15}\text{N}$ -labelled yeast protein, Mrs S. M. Hay for technical help, and the Grampian Health Board, Rank Prize Funds, Wyeth Laboratories, the MRC and the AFRC for financial support.

de Benoist, B., Abdulrazzak, Y., Brooke, O. G., Halliday, D. & Hillward, D. J. (1984). *Clinical Science* **66**, 155-642.

Pencharz, P. B., Farri, L. & Papageorgiou, A. (1983). *Clinical Science* **64**, 611-616.

Waterlow, J. C., Golden, M. H. N. & Garlick, P. J. (1978). *American Journal of Physiology*



**The effect of variety of spring barley straw and of ammonia treatment on nutritive value.** By E. A. LUFADJEU, G. A. BLACKET and E. R. ØRSKOV, Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB in collaboration with The North of Scotland College of Agriculture

In the many investigations of effect of chemical treatment on the nutritive value of straw, little emphasis has been given to difference between straws of different origin. It is generally accepted that wheat straw has a lower nutritive value than barley straw. Hartley *et al.* (1985), using *in vitro* techniques, showed differences between two varieties of wheat straw, and Kernan *et al.* (1979) found differences between varieties of wheat, oat and barley straw.

We therefore determined the nutritive value of 13 varieties of barley straw, grown and harvested under similar conditions, and investigated whether the responses to 30 g anhydrous ammonia/kg in sealed stacks for 4 weeks differed between varieties. The nylon-bag method as described by Ørskov *et al.* (1980) was used. Each sample was incubated in the rumen of three fistulated sheep fed on hay. A sample was withdrawn at either 8, 16, 24, 48 or 72 h of incubation, and the results fitted using the equation  $p = a + b(1 - e^{-ct})$  where  $p$  is degradability at time  $t$  and  $a$ ,  $b$  and  $c$  are constants.

Varieties	Untreated		Treated	
	Degradability		Degradability	
	48 h	Potential	48 h	Potential
Acclaim	55.4	73.3	65.6	74.8
Corgi	58.7	66.2	68.6	76.8
Delta	39.0	57.5	63.6	67.0
Doublet	60.6	66.6	73.6	83.9
Golden Promise	40.2	56.1	63.4	73.4
Golf	46.7	54.4	65.4	71.4
Heriot	54.9	61.2	72.6	84.0
Javelin	53.2	66.4	71.1	79.6
Midas	45.8	56.6	72.6	78.2
Nairn	51.1	56.4	67.4	70.4
Tasman	56.7	67.3	70.8	75.7
Triumph	50.1	62.2	68.5	72.2
Tweed	48.5	75.6	70.2	79.4
SE of differences	2.66	4.71	1.99	2.97

In the Table the results relating to the fitted value at 48 h disappearance of dry matter, and that of the asymptote of the equation defined as potential degradability, are given. There were substantial differences between the varieties of straw in nutritive value varying from 39.0 to 60.6 at 48 h, and from 54.4 to 75.6 in the potential degradability. The difference between the varieties when they were treated with ammonia were less with values varying from 63.4 to 73.6 for 48 h disappearance and from 70.4 to 84.0 in the potential degradabilities. The 48 h disappearances of untreated and treated straw were poorly correlated.

Hartley, R. D., Keene, A. S. & Deschard, G. (1985). In *International Conference on New Approaches to Research on Cereal Carbohydrates*, Carlsberg Research Centre, Copenhagen, Denmark. (In the Press.)

Kernan, J. A., Crowle, W. L., Spurr, D. T. & Coxworth, E. C. (1979). *Canadian Journal of Animal Science* **59**, 511.

Ørskov, E. R., Hovell, F. D. DeB. & Mould, F. (1980). *Tropical Animal Production* **5**,

**The use of particle-bound microbial enzymes to predict the rate of degradation of plant fibre in the rumen.** By AYONA T. SILVA, R. J. WALLACE and E. R. ØRSKOV, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

The rate of fibre degradation in the rumen can be estimated from the rate of weight loss of substrate incubated in a porous nylon bag *in situ* (Ørskov *et al.* 1980). Although this method is of great value in defining optimum conditions for cellulolysis, sometimes a greater sensitivity is required. The present study was therefore undertaken to investigate the possibility that measurement of the activity of particle-associated microbial enzymes might be used to assess the conditions of cellulose digestion in the rumen.

Eight rumen-cannulated sheep were individually penned and two animals were randomly allocated to each of four diets. Diets were prepared to achieve four different rates of fibre digestion. The diets consisted of (A) rolled barley, (B) rolled barley and hay in a proportion of 0.6:0.4, (C) hay, (D) molasses and hay in a proportion of 0.4:0.6. Barley straw was incubated in nylon bags for 24 and 48 h to estimate dry matter degradability (Ørskov *et al.* 1980). Separate nylon bags were incubated at the same time to determine the extent of microbial colonization from enzyme activities. Following incubation, the bags were washed thoroughly in water and enzymes were extracted from the washed fibres using carbon tetrachloride and lysozyme (Nossal & Heppel, 1966).

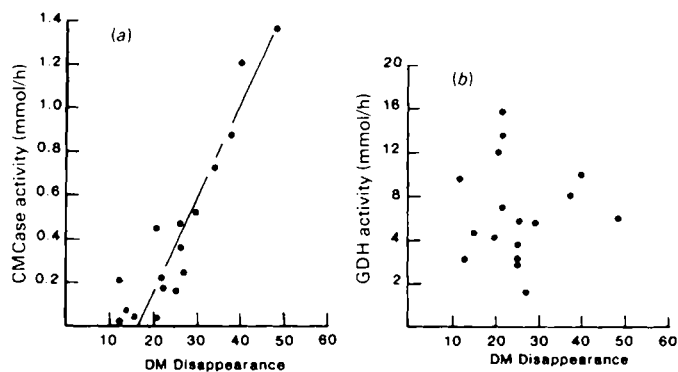


Fig. 1. The relation between particle-bound enzyme activities and dry matter disappearance of straw incubated in nylon bags for 24 h

Carboxymethyl cellulase (*EC* 3.2.1.4; CMCCase) activity was measured by the release of reducing sugars from carboxymethyl cellulose, and glutamate dehydrogenase (*EC* 1.4.1.2; GDH) was assayed by the rate of oxidation of NADH.

The relation between dry matter degradability and CMCCase was linear ( $r$  0.98; Fig. 1(a)), but particle-associated GDH activity bore no relation to degradability (Fig. 1(b)). The corresponding enzyme activities of straw which had not been incubated in the rumen were negligible.

The contrast between Fig. 1(a) and 1(b) probably reflects the fact that GDH is found in both cellulolytic and non-cellulolytic rumen bacteria whereas CMCCase is derived specifically from cellulolytic bacteria. Thus the CMCCase associated with plant fibres incubated in rumen fluid may provide a good measurement of the rate of dry matter degradation.

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**Changes in sensitivity and preferences for salt during dialysis.** By R. SHEPHERD and C. A. FARLEIGH, *AFRC Food Research Institute, Colney Lane, Norwich NR4 7UA* and S. JEVONS, *School of Health and Applied Sciences, Leeds Polytechnic, Leeds LS1 3HE* and J. S. PRYOR, *Norfolk and Norwich Hospital, Norwich NR1 3SR*

Patients with renal failure tend to have lower taste sensitivity than controls when assessed using solutions of primary tastants at threshold, and the sensitivity increases after dialysis (Burge *et al.* 1979). In the present experiment, patients were tested for changes, during dialysis, of both sensitivity and preference for salt in foods.

Twelve female patients, on sodium-restricted diets, were tested before and after dialysis, on three occasions with pea soup and with bread. They were presented with samples containing six concentrations of sodium chloride (1.04–7.86 g Na/kg soup; 1.51–11.85 g Na/kg bread). These were rated on a seven-category scale of intensity of saltiness, giving a measure of sensitivity and on a 100-mm graphic relative-to-ideal rating scale, which gives an estimate of the amount by which the sample differs from the patient's own 'ideal' (or most preferred) concentration (Shepherd *et al.* 1985).

The ratings for both scales were higher after dialysis, for both the bread and the soup. Regression lines were fitted to the ratings for each subject pre- and post-dialysis, and the slopes and ideal concentrations calculated. There were increases in the slopes of the regression lines and decreases in ideal concentrations on dialysis, as shown in the Table.

	Bread			Soup		
	Pre-dialysis	Post-dialysis	SE	Pre-dialysis	Post-dialysis	SE
Relative to ideal ratings:						
Average rating	-19.3 <sup>***</sup>	-11.8 <sup>***</sup>	1.5	-9.5 <sup>***</sup>	0.0 <sup>***</sup>	1.4
Slope of function	16.9*	20.8*	1.1	32.3†	35.1†	0.9
Log <sub>e</sub> (ideal)	8.03†	6.93†	0.4	5.94 <sup>***</sup>	5.65 <sup>***</sup>	0.04
'Ideal' (g Na/kg)	307	102	—	38	28	—
Intensity ratings:						
Average rating	2.12*	2.34*	0.07	2.40 <sup>***</sup>	2.80 <sup>***</sup>	0.06
Slope of function	0.82	0.88	0.07	1.22*	1.42*	0.05

† $P < 0.10$ , \* $P < 0.05$ , \*\*\* $P < 0.001$ .

The increase in overall ratings and the slopes of the functions may be interpreted as increases in sensitivity to salt taste, in agreement with Burge *et al.* (1979). There was an accompanying decrease in the most preferred concentration in the present study. The relation between such taste changes and biochemical changes during dialysis are being investigated. The lower sensitivity and higher preferences before dialysis may make compliance with Na-restricted diets more difficult for these patients.

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**Highly successful artificial rearing of rat pups with rats' milk or rats' milk/milk substitute combinations.** By J. L. SMART and J. TONKISS, *Department of Child Health, The Medical School, Oxford Road, Manchester M13 9PT*

The semi-automated artificial rearing technique for rat pups is potentially an extremely useful tool for developmental studies in many disciplines, including nutrition (Hall, 1975). However, it has proved difficult to achieve normal growth, due to the occurrence of abdominal distention ('bloat') when the rats are reared on substitute 'milks'. If 'milk' is infused in amounts similar to the milk intake of mother-reared (MR) rats, bloat is likely to ensue; if the infusion rate is reduced to avoid bloat, growth is deficient (Smart *et al.* 1984). We report the results of artificially-rearing rat pups on rats' milk, either alone or in combination with a milk substitute as described by Smart *et al.* (1984).

Milk was obtained from anaesthetized dams (Sagatal), stored at  $-20^{\circ}$ , then thawed, pooled, and homogenized by sonication. Artificial and normal rearing procedures are described by Smart *et al.* (1984). In Expt 1, rats' milk only was given from postnatal days 5 to 12. In Expt 2, two different regimens were tried from days 5 to 20, utilizing rats' milk (R) and a milk substitute (milk A of Smart *et al.* 1984; composition, g/l: protein 79, carbohydrate 36, fat 102). (i) Abrupt switch: R alone for days 5–12, then A alone till day 20. (ii) Gradual change: R alone for days 5–7, R plus A (3:1 v/v) for days 8–10, R plus A (1:1, v/v) for days 11–13, R plus A (1:3, v/v) for days 14–16, A alone for days 17–20.

At 12 d in Expt 1 and 20 d in Expt 2, rats were killed for autopsy and the following measurements taken: weights of whole body, brain, heart, liver, kidney, adrenals, gastrocnemius muscle, spleen, stomach, caecum and epididymal fat pads; lengths of body (nose–rump), small and large intestines. Numbers of pups were: Expt 1, five MR, five artificially-reared (AR); Expt 2, six MR, eight AR (i), seven AR (ii).

No pup developed bloat in either experiment and only one died (for non-nutritional reasons). In Expt 1, pups given rats' milk had significant deficits in body-weight (21%) and length and in the weights of brain, heart, spleen and epididymal fat pads, probably because the amounts infused were lower than those taken by MR pups. Infusion rates were increased in Expt 2 and both AR groups attained normal body-weight and length. Absolute measurements of most organs were normal (brain, kidney, gastrocnemius muscle, spleen and epididymal fat pad weights, large intestine length) or significantly greater than normal (stomach and caecum weights, small intestine length) in both groups. The exceptions were heart weight (low) in both groups, liver weight (high) in the gradually-changed group and adrenal weight (low) in the abruptly-switched group. This growth achievement was much better than any previously reported for AR rats.

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**Species comparisons of the effect of essential fatty acid deficiency on fatty acid composition of liver total phospholipids.** By S. C. CUNNANE, Y-S. HUANG, M. S. MANKU and D. F. HORROBIN, *Efamol Research Institute, Kentville, Nova Scotia, Canada B4N 4H8*

Substantial species differences in the composition of essential fatty acids (EFA) in various tissues have been reported (Crawford *et al.* 1976; Stone *et al.* 1979; Horrobin *et al.* 1984) which reflect differences in the desaturation of the parent EFA, 18:2n-6 and 18:3n-3. We therefore compared the effect of EFA deficiency on liver fatty acid composition of weanling Sprague-Dawley rats, Swiss-albino mice, Golden Syrian hamsters and Hartley guinea-pigs with particular interest in differences in the accumulation of 20:3n-9. All animals were given a fat-free semi-synthetic diet containing 100 g safflower oil/kg (EFA-adequate control group; CT) or 100 g medium-chain triglyceride/kg (EFA-deficient group; ED) for 10, 12, 16 or 23 weeks (rats, mice, hamsters and guinea-pigs respectively).

Livers were removed and the total phospholipid fatty acids analysed by gas-liquid chromatography.

		Liver fatty acid (%)							
		Rat (n8)		Hamster (n8)		Mouse (n8)		Guinea-pig (n8)	
Fatty acid	Diet	Mean	SD	Mean	SD	Mean	SD	Mean	SD
20:3n-9	CT	ND		ND		ND		ND	
	ED	19.8	1.2	3.9	0.4	3.5	0.2	3.7	0.4
18:2n-6	CT	9.5	0.8	19.8	2.0	18.2	1.9	35.7	1.9
	ED	2.8*	0.6	8.8*	3.4	10.8	2.4	8.3*	1.7
20:4n-6	CT	35.6	1.0	17.0	1.2	24.9	0.8	6.7	0.6
	ED	10.9*	1.7	8.5*	1.3	16.7*	2.3	3.1*	0.3
29:3n-9/20:4n-6	ED	1.82		0.46		0.21		1.19	

ND, not detected. \* $P < 0.05$ .

Two points are of interest: (1) 20:3n-9 accumulation was greatest in the rat even though it was given the EFA-deficient diet for the shortest period of time, and (2) although the mice were given the EFA-deficient diet for 12 weeks, 20:3n-9/20:4n-6 was still  $< 0.4$ , the cut-off for EFA deficiency. Since 20:3n-9 is a desaturation/elongation product of 18:1n-9 equivalent to arachidonic acid (20:4n-6), it is suggested that the higher accumulation of 20:3n-9 in the rat was due to higher activities of the associated enzymes than in the hamster, mouse or guinea-pig. It follows that the relative increase in desaturase activity in EFA deficiency is species dependent, being greater in the rat than in the other species studied.

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**The percentage of arachidonic acid in triglyceride is inversely related to total triglyceride: indication of an essential fatty acid-rich pool of triglyceride.** By S. C. CUNNANE, *Efamol Research Institute, Kentville, Nova Scotia, Canada B4N 4H8*

In studies of essential fatty acid (EFA) composition, a negative correlation has been observed between the percentage of arachidonic acid (20:4n-6) in triglyceride (TG) and the total amount of TG in liver or plasma. This relation has been found in studies using mice, hamsters and rats under conditions of EFA, magnesium and pyridoxine deficiencies, starvation, and ethanol or carbon tetrachloride feeding over a wide range of TG values ( $n$  150). The correlation averages  $-0.70$  (varies from  $-0.43$  in hamster plasma to  $-0.76$  in rat plasma). One possible explanation for this relation is the existence of a class of TG which contains a higher proportion of EFA, particularly 20:4n-6, than does the rest, which responds slowly to dietary influences on total TG mass. Thus, increases in liver or plasma total TG are composed mainly of 16:0, 18:0, 18:1n-9 and 18:2n-6 which proportionally reduces 20:4n-6 and vice versa. This relation was further studied by analysing TG-class fatty acid composition in two groups of mice in which differences in liver total TG were maximized.

To increase liver total TG, one group was given 50  $\mu$ l CCl<sub>4</sub> in paraffin and killed 24 h later. To reduce liver total TG, the other group was given restricted access to food for 10 d. TG classes were separated according to double-bond composition using silver nitrate thin-layer chromatography, and their fatty acid compositions compared.

In mice treated with CCl<sub>4</sub> (high liver total TG, lower percentage 20:4n-6), 20:4n-6 was located mainly in the band corresponding to 6 double bonds (3.7% of total fatty acid composition) but was also found in bands corresponding to 4 and 5 double bonds (2%). In the starved mice (low liver total TG, high percentage 20:4n-6), 20:4n-6 was located in the bands corresponding to 5 and 6 double bonds (6%). With the exception of 18:3n-3, no EFA with more than 2 double bonds was located in a band representing less than 4 double bonds. Thus, fatty acids located in the bands representing 0-4 double bonds did not contain EFA other than 18:2n-6 and a small percentage of 18:3n-3. These results suggest that, in liver and plasma, 20:4n-6 is concentrated in a class of TG which is EFA-rich. The EFA do not appear to be distributed in all the TG classes but are located in double-bond rich TG only. If TG is a significant source of 20:4n-6 for postaglandin synthesis, this sub-class of EFA-rich TG may be an important source.

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